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Relative Potencies for Acute Effects of Pyrethroids on Motor Function in Rats

M. J. Wolansky,* C. Gennings,† and K. M. Crofton‡:[1]

*National Research Council, Research Triangle Park, North Carolina 27711; †Solveritas, LLC, Virginia Bio-Technology Research Park, Richmond VA 23219; and ‡Neurotoxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

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The prevalence of pyrethroids in insecticide formulations has increased in the last decade. A common mode-of-action has been proposed for pyrethroids based on *in vitro* studies, which includes alterations in sodium channel dynamics in nervous system tissues, consequent disturbance of membrane polarization, and abnormal discharge in targeted neurons. The objective of this work was to characterize individual dose-response curves for *in vivo* motor function and calculate relative potencies for eleven commonly used pyrethroids. Acute oral dose-response functions were determined in adult male Long Evans rats for five Type I (bifenthrin, S-bioallethrin, permethrin, resmethrin, tefluthrin), five Type II (β-cyfluthrin, λ-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate) and one mixed Type I/II (fenpropathrin) pyrethroids (n = 8–18 per dose; 6–11 dose levels per chemical, vehicle = corn oil, at 1 ml/kg). Motor function was measured using figure-8 mazes. Animals were tested for 1 h during the period of peak effects. All pyrethroids, regardless of structural class, produced dose-dependent decreases in motor activity. Relative potencies were calculated based on the computed ED30s. Deltamethrin, with an ED30 of 2.51 mg/kg, was chosen as the index chemical. Relative potency ratios ranged from 0.009 (resmethrin) to 2.092 (esfenvalerate). Additional work with environmentally-based mixtures is needed to test the hypothesis of dose-additivity of pyrethroids.

Key Words: **pyrethroids; motor function, relative potency.**

Pyrethrins are derived from the flowers of *Chrysanthemum cinerariaefolium* that have been used as insecticides for more than a century (LaForge and Markwood, 1938). Pyrethroids are structural derivatives of pyrethrins that have greater potency and environmental stability (Casida, 1980; Elliott, 1978). Pyrethroids and pyrethrins are used in a wide array of indoor and outdoor applications, including medicinal, veterinary, and agricultural usages (ATSDR, 2003). Pyrethroid usage has been estimated at 23% of the worldwide insecticide market (Casida and Quistad, 1998). Agricultural and home use of pyrethroids is increasing, partly due to phase-outs of older insecticides (Amweg *et al.*, 2005).

Pyrethroids are classified as Type I or Type II according to both chemical structure and biological effects of high-dose acute exposures (Gammon *et al.*, 1981; Gray, 1985; Lawrence and Casida, 1982; Verschoyle and Aldridge, 1972, 1980). Compounds lacking an α-cyano group on the phenoxybenzyl moiety produce toxic signs characterized by aggressive sparring and tremors (Type I, or T-syndrome). The presence of an α-cyano group on the phenoxybenzyl moiety leads to a syndrome characterized by choreoathetosis and salivation (Type II, or CS-syndrome). There are a few compounds with mixed signs, including both tremors and salivation (Gammon *et al.*, 1981; Lawrence and Casida, 1982; Verschoyle and Aldridge, 1980). Accordingly, these compounds have been labeled Type I/II or TS.

Pyrethroids act primarily on the nervous system. The commonly accepted mechanism-of-action of pyrethroids is the prolongation of the open state of voltage-dependent sodium channels in nervous tissue (Narahashi, 2000; Soderlund *et al.*, 2002; Vijverberg and van den Bercken, 1990). These altered sodium channels result in repetitive firing or depolarizing block of the neuron, depending on how long the channel open state is prolonged (Soderlund *et al.*, 2002; Narahashi, 2000). Other channel and receptor systems in neuronal tissues have been proposed to play a role in the generation of compound-specific clinical symptoms in mammals, including calcium channels and $GABA_A$ receptors (Crofton and Reiter, 1987; Hildebrand *et al.*, 2004; Lawrence and Casida, 1983; Soderlund *et al.*, 2002). However, the role of these other channels or receptors in the action of pyrethroids is not well established (Ogata *et al.*, 1988; Shafer and Meyer, 2004; Soderlund *et al.*, 2002).

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[1] To whom correspondence should be addressed at Neurotoxicology Division, MD: B105–04; National Health and Environmental Effects Research Laboratory, ORD, U.S. EPA, Research Triangle Park, NC 27711. E-mail: crofton.kevin@epa.gov.

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The Food Quality Protection Act (FQPA), promulgated in 1996, requires the U.S. EPA to consider the cumulative toxicity of pesticides having a common mode-of-action. Risk-assessment approaches to additivity assume, where data are lacking, that chemicals with similar modes-of-action act in a dose-additive fashion (U.S. EPA, 1986, 2000). There are currently no published data on the additivity, or lack thereof, for pyrethroids. In addition, there is debate about whether a common mode-of-action exists for all pyrethroids (Soderlund *et al.*, 2002). Research needs to reduce uncertainty in pyrethroid cumulative risk assessments include: (1) dose-response functions for individual pyrethroids, (2) calculation of relative potencies, and (3) studies that test additivity of relevant mixtures of pyrethroids. This paper addressed the first two items. We characterized dose-responses for the acute effects of 11 pyrethroids on motor function. A simple assessment of motor activity was used, as this behavior has been extensively characterized for a number of pyrethroids (Crofton *et al.*, 1995; Crofton and Reiter, 1984, 1988; Hornychova *et al.*, 1995; Hoy *et al.*, 2000; McDaniel and Moser, 1993). These motor activity data were analyzed using a nonlinear exponential threshold model (Casey *et al.*, 2004) to estimate ED30s (effective dose that produces a 30% decrease in activity) and threshold doses (the highest dose that has no effect on activity). The ED30s were used to calculate relative potencies (Safe, 1998; U.S. EPA, 2000; Villeneuve *et al.*, 2000; Wilkinson *et al.*, 2000). These data will be utilized in future work testing the assumption of dose-additivity for environmentally relevant mixtures of pyrethroids.

MATERIALS AND METHODS

Subjects. Male Long Evans rats (CRL, Wilmington, MA) were obtained at 55–57 days of age, and were housed two per cage in standard polycarbonate hanging cages (45 cm × 24 cm × 20 cm) containing heat-sterilized pine shavings (Beta Chips, Northeastern Products, Inc., Warrensburg, NY). All animals were given a 5–9 day acclimation period and were maintained on a 12:12 h photoperiod (0600:1800). Food (Purina 5001 Lab Chow) and tap water were provided *ad libitum*. Colony rooms were maintained at 22.0 ± 2.0°C and relative humidity at 55 ± 20%. The facility is approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All experimental protocols were approved in advance by the National Health and Environmental Effects Research Laboratory's Animal Care and Use Committee.

Chemicals. Eleven pyrethroids were tested. Table 1 lists the common names, chemical formulas, isomer composition, purity, molecular weights, LD50s, and the number of doses and dose ranges for each pyrethroid. Doses were calculated based on percent active ingredient in the technical product (purity is listed in Table 1). Pesticides were kindly supplied by their manufacturers: permethrin, bifenthrin, and cypermethrin (FMC Corporation, Philadelphia, PA); esfenvalerate (Dupont Crop Protection, Wilmington, DE); deltamethrin and β-cyfluthrin (Bayer Cropscience, Research Triangle Park, NC); tefluthrin and λ-cyhalothrin (Syngenta Crop Protection, Greensboro, NC); and fenpropathrin, resmethrin, and S-bioallethrin (Valent USA Corporation, Walnut Creek, CA). Note that these pyrethroids were from the same lot # (or an equivalent lot having similar purity and isomer composition) as those used in the manufacturer-sponsored studies summarized in Soderlund *et al.* (2002). Pyrethroid dosing solutions were prepared daily by dissolving in corn oil (Sigma, Co., USA). An exception was β-cyfluthrin, which was first dissolved in a small volume of acetone; then a measured volume of corn oil was added to obtain a stock solution based on final volume, with serial dilutions used to prepare final dose concentrations. Acetone was allowed to vaporize overnight within a fume hood in the dark before the solution was used the following morning. High concentrations of resmethrin (≥400 mg/ml) gradually precipitated over the course of a few hours; therefore these solutions were intermittently stirred and gently heated (40–50°C) to maintain solubility. Dosing solutions were used at room temperature.

Animal treatment. Pyrethroids were administered by gavage in 1 ml/kg corn oil. Six to eleven doses were examined per compound, with dose groups balanced for time-of-day and test chamber. Dose selection was based on pilot studies, with the goal to have at least three no-effect levels. Prior to dosing, animals were moved from the colony room to an isolated dosing room within the testing laboratory. After a minimum 1 h acclimation, animals were removed from home cages, dosed, and then returned to the home cages until testing. Dose levels inducing excessive toxicity (i.e., leading to prolonged Type I or II clinical signs, or mortality) were not included in final experiments. This was done to ensure estimations of alterations in general motor function and not decreases due to excessive toxicity. For most cases, 8–18 animals per group were tested. Each experiment was divided into at least two blocks. Control animals (vehicle only) were included in each block. Nonintubated animals were included in some of the initial experiments to ensure a lack of effect of the vehicle and intubation procedures. All rats were randomly assigned to treatment groups and to individual mazes. Independent groups of rats were used for each experiment.

Motor activity. Rats were placed into individual plastic cages with pine shavings and allowed to acclimate to the test room, which was maintained at the same environmental conditions as the animal colony and dosing room, for 5 min before being tested. Motor activity was measured for 1 h using 16 figure-eight mazes, each consisting of a series of interconnected alleys (10 × 10 cm) converging on a central arena and covered with transparent acrylic plastic (Norton *et al.*, 1975; Reiter *et al.*, 1975). Horizontal and vertical activity were detected by photo-transistor/photodiode pairs, eight equally spaced around the mazes at 0.5 in. above the floor (horizontal), and four pairs located 3 in. above the floor in the central arena. Photodetectors were sampled at a 1-kHz rate, and each time a photobeam was interrupted, an activity count was registered. Total activity was calculated as the sum of horizontal and vertical activity counts. Photobeam calibration was checked daily prior to testing. Maze assignments, order of testing, and time of day were counterbalanced across treatment groups. All testing was conducted between 0900 and 1700 h.

Testing was conducted at the time of peak effects. The time of peak effect for some pyrethroids was selected from previous work done under similar dosing and motor activity testing conditions (Crofton and Reiter, 1984, 1988; McDaniel and Moser, 1993). For other compounds, the time of peak effects was obtained from pilot time-course studies using motor activity testing or behavioral observations. Time from dosing to testing was as follows: 1 h, S-bioallethrin; 1.5 h, permethrin, cypermethrin; 2 h, β-cyfluthrin, esfenvalerate, deltamethrin, tefluthrin and fenpropathrin; 2.5 h, λ-cyhalothrin; and 4 h, bifenthrin, resmethrin. All animals were observed before and after motor activity testing for signs of excessive toxicity.

Statistical analysis. Activity data were analyzed using a nonlinear exponential threshold additivity model (Casey *et al.*, 2004). The model is algebraically equivalent to the definition of additivity (i.e., zero interaction) given by Berenbaum (1985) and can be related to the isobologram for a combination of chemicals (Loewe, 1953) through the interaction index. This is the model that will be used to analyze data from future mixtures research. The method of maximum quasi-likelihood was used to estimate model parameters. The adequacy of the fit of the additivity model to the single chemical data was assessed graphically and through goodness-of-fit statistics. This additivity model was used to determine the dose associated with a 30% decrease in motor activity (ED30) for each pyrethroid. Approximate 95%

TABLE 1
Physical, Chemical and Biological Features of Type I, Type I/II, and Type II Pyrethroids Evaluated in this Work

Common name	Chemical formula	Isomer composition*	Type	Purity	MW	LD50** (mg/kg)	Number of doses & dose range (mg/kg)
Deltamethrin	(*S*)-cyano-(3-phenoxyphenyl)methyl (1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate	100% (1R,3R, alphaS)	II	98.9	505.2	66.7[d]	6, 0.03–10
Cypermethrin	(*R,S*)-cyano-(3-phenoxyphenyl)methyl (1*R,S*)-*cis-trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	48.7% cis, 51.3% trans mixture of all 8 isomers	II	88.0	416.3	250[a]	6, 0.1–120
β-Cyfluthrin	(*R,S*)-cyano-(4-fluoro-3-phenoxy-phenyl)methyl-(1*RS*)-*cis,trans*-3-(2,2-ichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate	≤2% (1R,3R,αR+1S,3S,αS) 30–40% (1R,3R,αS+1S,3S,αR) ≤3% (1R,3S,αR+1S,3R,αS) 57–67% (1R,3S,αS+1S,3R,αR)	II	99.2	434.3	77[c]	8, 0.05–15
Esfenvalerate	(*S*)-cyano-(3-phenoxyphenyl)methyl (1*S*)-2-(4-chlorophenyl)-3-methylbutanoate	85.5% *SS isomer* 12.0% SR, RR, RS 2.5% other inerts	II	98.6	419.9	87[a]	6, 0.03–10
λ-Cyhalothrin	(R,*S*)-cyano-(3-phenoxyphenyl) methyl-(Z)-(1*R,S*)-*cis*-3-(2-chloro-3,3,3-trifluoro-prop-1-enyl)-2,2-dimethylcyclopropanecarboxylate	50% (S-α-cyano, Z-1R-cis) 50% (R-α-cyano-Z-1S-cis)	II	87.7	449.9	56[a]	9, 0.015–15
Fenpropathrin	(*R,S*)-cyano-(3-phenoxyphenyl)methyl 2,2,3,3-tetramethylcyclopropanecarboxylate	50% S-cyano 50% R-cyano	I/II	91.8	349.4	66[a]	8, 0.01–24
Resmethrin	(5-benzyl-3-furyl)methyl (1*RS*)-*cis-trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)-cyclopropanecarboxylate	30% cis, 70% trans 1:1 ratio of 1R, 1S	I	92.3	338.4	2,000[a]	11, 0.5–900
S-Bioallethrin	(*S*)-3-allyl-2-methyl-4-oxocyclopent-2-enyl (1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)-cyclopropanecarboxylate	92.1% (d-trans-d) 5.6% (d-trans-l) 1.7% (l-trans-d,l) <1% (d,l-cis-d,l)	I	95.6	302.4	700[a]	7, 0.5–150
Permethrin	3-phenoxybenzyl (1*R,S*)-*cis-trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate	40%cis, 60%trans 1:1 ratio of 1R, 1S	I	92.0	391.3	1,200[b]	9, 0.1–200
Bifenthrin	2-methylbiphenyl-3-ylmethyl (Z)-(1*R*)-*cis*-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cypropanecarboxylate	100% (Z, 1R cis)	I	89.0	422.9	55[a]	9, 0.03–28
Tefluthrin	2,3,5,6-tetrafluoro-4-methylbenzyl (Z)-(1*R*)-*cis*-3-(2-chloro-3,3,3-trifluoro-prop-1-enyl)-2,2-dimethyl-cypropanecarboxylate	100% (Z, 1R cis)	I	92.6	418.7	22[a]	8, 0.01–12

*Isomer composition of the technical product expressed as a percentage of the purity from column 5. Information supplied by the manufacturer.
[a]LD50 values were taken from WHO (1996).
[b]LD50 values were taken from McGregor (1999).
[c]LD50 values were taken from EC_HCPDG, 2002.
[d]LD50 values were taken from USEPA Federal Register, Sept 25, vol. 62, no.186 (1997).

confidence intervals were computed for the ED30s by applying the delta method. The nonlinear exponential threshold additivity model was also used to obtain the threshold dose and its 95% confidence intervals for each single compound. This threshold dose represents an estimate of the highest no-effect dose level at which treated rats would not display any decrease in motor activity. Relative potencies were calculated from ED30s using deltamethrin as the index chemical. Deltamethrin was chosen due to its extensive toxicological database and the reliability of effects on motor activity within this laboratory (Crofton *et al.*, 1995; Crofton and Reiter, 1984, 1987; Gilbert *et al.*, 1990).

RESULTS

Pilot studies provided dose ranges, excluding excessive toxicity, for use in the formal dose-response experiments. In the formal studies, no signs of excessive toxicity for most of the pyrethroids were observed with cage-side observations conducted before and after motor activity testing. Only in the case of tefluthrin (12 mg/kg), fenpropathrin (24 mg/kg), and permethrin (200 mg/kg) were excessive signs (i.e., prolonged (>4 h) clinical

signs, or clear and complete Type I or Type II syndromes) seen at the highest doses and only with a small percentage of animals. These doses were not used in any data analyses.

All pyrethroids induced dose-dependent decreases in motor activity (Fig. 1). ED30s and threshold doses are listed in Table 2. ED30s varied by more than two orders of magnitude (Table 2) from the least (resmethrin) to the most potent (esfenvalerate) compound. Threshold doses for the eleven pyrethroids were approximately 39% of the ED30s (Table 2). The predictability of the model for each of the single chemical data showed significant Spearman's rho coefficients in all cases (mean ρ = 0.68 range: 0.5–0.81). Table 3 lists estimated model parameters. All estimated parameters were significant. Relative potencies, calculated as ratio of the ED30 for the index chemical, deltamethrin (ED30 = 2.51 mg/kg), over each chemical's ED30, are seen in Table 4.

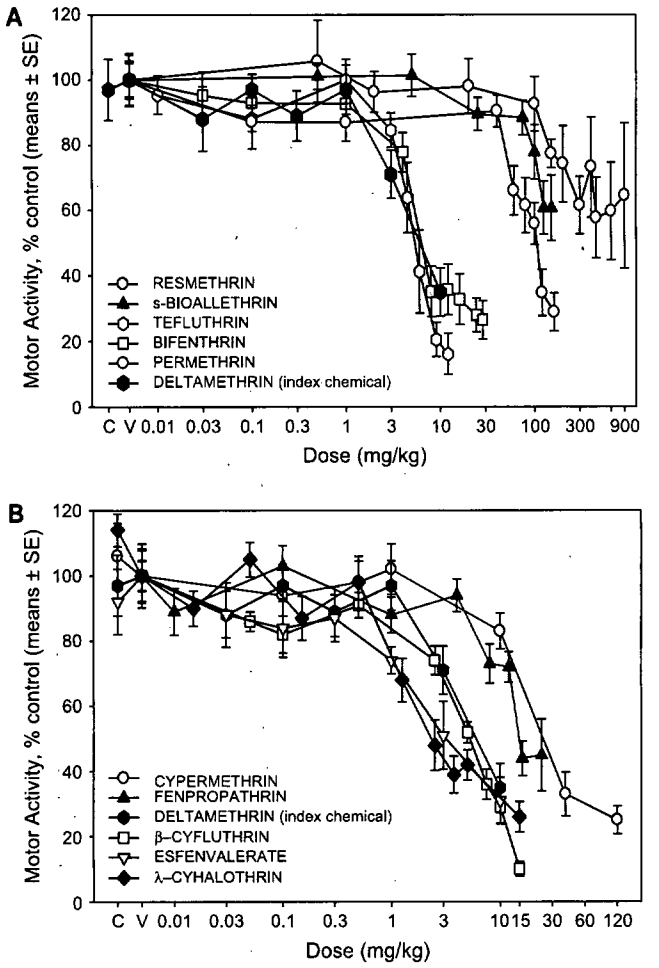

FIG. 1. Dose-response curves for the examined compounds. (A) Five Type I pyrethroids. B) Five Type II pyrethroids and the mixed Type I/II, fenpropathrin. Deltamethrin, the index chemical, is also included in both graphs. Data are expressed as percentage of each respective vehicle control. The X-axis is expressed in *log* scale. (C = nonintubated control; V = corn oil vehicle control; n = 8–18 per dose-group).

DISCUSSION

Motor activity was used as an endpoint to determine dose-response relationships after acute exposure to each of eleven pyrethroid insecticides. All pyrethroids tested produced dose-dependent decreases in motor activity. Relative potencies of the eleven pyrethroids spanned more than two orders of magnitude. The present findings confirm the generality of the motor-depressant effect of pyrethroids, expand these to include other pyrethroids, and more importantly, provide extensive dose-response data for each chemical.

Dose-dependent decreases in motor activity are consistent with a wide variety of previous reports on the acute effects of pyrethroids. Past work from this laboratory and others (Crofton *et al.*, 1995; Crofton and Reiter, 1984, 1987, 1988; Gilbert *et al.*, 1990; McDaniel and Moser, 1993) demonstrated decreased motor activity for five of the currently tested compounds using the same test method (i.e., figure-eight mazes). Decreases in motor activity have been demonstrated in other testing devices after acute or short-term exposure, including permethrin (Hoy *et al.*, 2000), fenvalerate (De Souza Spinosa *et al.*, 1999), cyhalothrin (Righi and Palermo-Neto, 2003), λ-cyhalothrin (Hornychova *et al.*, 1995; Ratnasooriya *et al.*, 2002), and cypermethrin (Hornychova *et al.*, 1995). The acute motor depressant effect has been also observed in mice after oral exposure to fenvalerate (Mandhane and Chopde, 1997) and deltamethrin (Chanh *et al.*, 1984).

There are a few reports of acute pyrethroid exposures resulting in increased motor activity. Increased motor activity was reported in mice acutely exposed to commercial formulations of fenvalerate and permethrin (Mitchell *et al.*, 1988). Husain *et al.* (1996) found an increase in motor activity 1 day after a 15-day exposure to a deltamethrin formulation. These reports of increased activity are difficult to interpret and may have resulted from unknowns in the commercial formulations used. Mitchell *et al.* (1988) used commercial formulations where the pyrethroids were 30% or less of the administered product. Husain *et al.* (1996) used Decis®, an emulsifiable formulation containing only 2.8% deltamethrin. Another report found no effect on motor activity in rats monitored using an automated open field following acute deltamethrin exposure (10 mg/kg) using a commercial formulation (Dayal *et al.*, 2003). This negative finding may be due to use of a commercial product containing only 2.8% deltamethrin (Dayal *et al.*, 2003), making the dose of deltamethrin used only ~0.28 mg/kg. This dose falls well below the threshold dose (0.99 mg/kg) in the current study. The importance of precise reporting of the exact nature of the tested agent, as well as the potential confounds that result from other components in commercial formulations, has been previously noted (Shafer and Meyer, 2004; Shafer *et al.*, 2005).

Potency estimates (ED30s) for the eleven pyrethroids ranged from 1.2 mg/kg for esfenvalerate to 292.8 mg/kg for resmethrin. Using deltamethrin as an index chemical, relative potencies

TABLE 2
ED30s and Threshold Doses for the Acute Effects of Eleven Pyrethroids on Motor Activity

Pyrethroid	Type	ED30*	95% Confidence intervals	Threshold dose*	95% Confidence intervals
Esfenvalerate	II	1.20 ± 0.14	[0.92, 1.47]	0.48 ± 0.12	[0.24, 0.71]
λ-Cyhalothrin	II	1.32 ± 0.13	[1.06, 1.57]	0.52 ± 0.13	[0.28, 0.77]
β-Cyfluthrin	II	2.21 ± 0.20	[1.80, 2.61]	0.88 ± 0.21	[0.47, 1.28]
Deltamethrin	II	2.51 ± 0.29	[1.94, 3.07]	0.99 ± 0.25	[0.51, 1.48]
Tefluthrin	I	2.26 ± 0.22	[1.84, 2.68]	0.90 ± 0.21	[0.49, 1.31]
Bifenthrin	I	3.21 ± 0.32	[2.59, 3.83]	1.28 ± 0.31	[0.67, 1.88]
Fenpropathrin	I/II	7.70 ± 0.65	[6.42, 8.97]	3.06 ± 0.67	[1.76, 4.37]
Cypermethrin	II	10.70 ± 1.34	[8.06, 13.34]	4.26 ± 1.14	[2.03, 6.49]
Permethrin	I	42.66 ± 3.58	[35.60, 49.70]	16.99 ± 3.82	[9.50, 24.48]
s-Bioallethrin	I	90.48 ± 8.05	[74.70, 106.30]	36.02 ± 7.91	[20.50, 51.54]
Resmethrin	I	292.80 ± 24.19	[245.40, 340.30]	116.60 ± 24.84	[67.84, 165.34]

*Values are ED30 (±SE) and Threshold Dose (±SE) in mg/kg. ED30 = dose (mg/kg) required to induce a 30% decrease in total motor activity in figure-eight maze as compared to the corresponding vehicle-treated control group. The threshold dose was defined as the highest no-effect dose level at which treated rats would respond with 100% control performance. ED30 and threshold dose estimates were obtained by fitting the single pyrethroid dose-response data, using a nonlinear exponential threshold additivity model (see Methods section for details).

ranged from 0.009 (resmethrin) to 2.092 (esfenvalerate). The model applied to fit the dose-response datasets also computed a threshold dose for each pyrethroid. Threshold doses for the eleven pyrethroids followed the same potency relationships calculated for the ED30s. This wide range of relative potencies is likely caused by a number of toxicokinetic and toxicodynamic factors. The presence of an α-cyano group on the alcohol moiety of the pyrethroid confers increased potency in both insects and mammals (Soderlund *et al.*, 2002; Valentine, 1990). This is evident comparing permethrin and cypermethrin (Tables 2 and 4). Another important factor in potency is the enrichment of active isomers in the technical product used (Glickman and Casida, 1982; Verschoyle and Barnes, 1972). Pyrethroid structures include chiral carbons (usually two to three). The activity of these analogs is highly dependent on the stereoisomeric configuration of the molecule and the rate of degradation by metabolizing enzymes (Glickman and Casida, 1982). Pyrethroids in the *cis*- configurations (i.e., deltamethrin, λ-cyhalothrin, tefluthrin, and bifenthrin) are more potent than those in the *trans*- configuration (Verschoyle and Aldridge, 1980). Thus, the applicability of relative potencies will be dependent on the isomeric composition of the test material.

The use of the relative potencies reported here to calculate cumulative risks should be tempered by uncertainties. The first uncertainty is that the relative potencies for motor activity may not predict all behavioral effects of pyrethroids. With only a few exceptions, high doses (i.e., lethal) of pyrethroids are well known to produce two very different syndromes of toxicity (Verschoyle and Aldridge, 1980). In addition, the acoustic startle response, a simple sensory-evoked motor reflex (Davis *et al.*, 1982), is differentially affected by some pyrethroids. Depending on structure, pyrethroids may increase or decrease this reflex behavior (Crofton and Reiter, 1984, 1988; Hijzen *et al.*, 1988; Hijzen and Slangen, 1988). Changes in motor activity have long been used in risk assessment and critically evaluated concerning specificity and reliability (Crofton *et al.*, 1991; Gerber and O'Shaughnessy, 1986; Kulig *et al.*, 1996; MacPhail *et al.*, 1989; Reiter and MacPhail, 1982; Stanton, 1994). Motor activity, like many behavioral functions, can be altered in both humans and laboratory animals by a wide variety of drugs and toxicants (Crofton *et al.*, 1991; MacPhail *et al.*, 1989; Tyron, 1985). Another uncertainty in the use of the present data in cumulative risk derives from the lack of data on the combined action of pyrethroid insecticides on any aspects of nervous system function. Predicting the effects of mixtures based on data from individual chemicals is difficult (Borgert

TABLE 3
Parameter Estimates from the Nonlinear Exponential Threshold Additivity Model (Casey *et al.*, 2004)

Parameters*	Estimates	SE	*p*-value
α	0.2521	0.0219	<0.001
β_1 (cyfluthrin)	−0.2686	0.0360	<0.001
β_2 (bifenthrin)	−0.1847	0.0275	<0.001
β_3 (bioallethrin)	−0.0066	0.0011	<0.001
β_4 (cypermethrin)	−0.0554	0.0117	<0.001
β_5 (deltamethrin)	−0.2364	0.0491	<0.001
β_6 (esfenvalerate)	−0.4959	0.0960	<0.001
β_7 (fenpropathrin)	−0.0770	0.0110	<0.001
β_8 (cyhalothrin)	−0.4505	0.0658	<0.001
β_9 (permethrin)	−0.0139	0.0016	<0.001
β_{10} (resmethrin)	−0.0020	0.0003	<0.001
β_{11} (tefluthrin)	−0.2621	0.0395	<0.001
δ	−0.2359	0.0681	<0.001

Note. The slope parameters (β) for the eleven single chemicals were negative and significant, indicating that, as the dose of the chemical increases, the mean motor activity decreases.

*α is the maximum effect parameter, β_i are the slope parameters for the individual chemicals (i = 1, ..., 11), and δ is the threshold parameter. See Casey *et al.* (2004) for model details. SE = standard error of the estimate.

TABLE 4
Relative Potencies for the Effects of Eleven Pyrethroids on Motor Activity

Pyrethroid	Relative potency
Deltamethrin	1.000
Esfenvalerate	2.092
λ-Cyhalothrin	1.902
β-Cyfluthrin	1.136
Tefluthrin	1.111
Bifenthrin	0.782
Fenpropathrin	0.326
Cypermethrin	0.235
Permethrin	0.059
s-Bioallethrin	0.028
Resmethrin	0.009

Note. Relative potencies for the effects of eleven pyrethroids on motor activity based on deltamethrin (ED30 = 2.51 mg/kg) as the index chemical. Relative potency was calculated as the ratio of the ED30 for deltamethrin over the ED30 for each chemical.

et al., 2004; Teuschler *et al.*, 2002; Wilkinson *et al.*, 2000). Concurrent exposures may interfere with the metabolism and kinetics of each individual chemical and/or its metabolites (Aldridge, 1990; Wilkinson *et al.*, 2000). Pyrethroid metabolic pathways have both common and compound-specific steps (Roberts and Hutson, 1998), and the toxicity of pyrethroid metabolites remains poorly evaluated (Beres *et al.*, 2000; NRCC, 1986). In addition, the severity of the effects of pyrethroids is influenced by route of exposure, vehicle, and dosing volume (Crofton *et al.*, 1995; Nishimura *et al.*, 1984; Soderlund *et al.*, 2002; Verschoyle and Aldridge, 1980). Furthermore, these acute data may or may not predict effects of longer-term exposures. Extrapolation of acute neurotoxicity findings can be difficult on both the quantitative and qualitative level (Bass *et al.*, 1985). Thus, the assumption of dose-additivity should be empirically tested before ruling out antagonistic or synergistic effects (Borgert *et al.*, 2004). Future work should include assessing other behavioral endpoints, and testing the hypothesis of additivity for mixtures of pyrethroids.

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Note: All raw data used in this manuscript are freely available for alternative analyses. Please direct requests to the corresponding author.

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